Regulatory Toxicology and Pharmacology 91 (2017) 165–172

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

# Derivation of endogenous equivalent values to support risk assessment and risk management decisions for an endogenous carcinogen: Ethylene oxide

C.R. Kirman*, S.M. Hays

*Summit Toxicology, P.O. Box 3209, Bozeman, MT 59715, United States*

ARTICLE INFO

*Keywords:*
Risk assessment
Risk management
Endogenous exposure
Biomarkers of exposure
Ethylene oxide
Point of departure

ABSTRACT

An approach is presented for ethylene oxide (EO) to derive endogenous equivalent (EE) values, which are endogenous levels normally found within the body expressed in terms of exogenous exposures. EE values can be used to support risk assessment and risk management decisions for chemicals such as EO that have both endogenous and exogenous exposure pathways. EE values were derived using a meta-analysis of data from the published literature characterizing the distribution for an EO biomarker of exposure, hemoglobin N-(2-hydroxyethyl)-valine (HEV), in unexposed populations. These levels are compared to the those reported in exposed populations (smokers, workers). Correlation between the biomarker of exposure and external exposures of EO were applied to this distribution to determine corresponding EE values, which range from 0.13 to 6.9 ppb for EO in air. These values are orders of magnitude higher than risk-based concentration values derived for EO using default methods, and are provided as a pragmatic, data-driven alternative approach to managing the potential risks from exogenous exposures to EO.

## 1. Introduction

Assessing and managing the potential risks associated with chemicals that have both exogenous and endogenous exposure pathways pose a challenge to the risk assessment community. For example, ethylene oxide (EO, CAS RN 75-21-8) has been identified as a carcinogen based upon increases in multiple tumor types in laboratory rodents (hematopoietic/lymphopoietic system, brain, lung, uterus, and peritoneal cavity; Snellings et al., 1984; Lynch et al., 1984; NTP, 1987), and increases in specific cancers in highly exposed workers (hematopoietic cancers, and possibly breast cancer; Steenland et al., 2003, 2004; Teta et al., 1999). EO is used in the production of chemicals and polymers, and as a disinfectant (e.g., sterilization medical equipment). Accordingly, exogenous exposures to EO can occur to workers in industries that make or use this chemical. However, EO is also produced endogenously in the body due to oxidation of ethylene, which is produced from the oxidation of lipid, methionine, and hemoglobin, and from intestinal bacteria (Wu et al., 2011; Liberman and Mapson, 1964; Sagai and Ichinose, 1980; Clemens et al., 1983; Shen et al., 1989; Tornqvist et al., 1989; Kessler and Remmer, 1990). The relative contribution of exogenous and endogenous pathways to total exposure, as well as their contribution to potential risks, is typically not well characterized.

This problem shares some of the same issues associated with assessing the potential risk from exposure to metals that also occur naturally in environmental media. Standard risk assessment practices can result in the derivation of risk-based concentrations for a chemical in environmental media that are below naturally occurring background levels. For example, naturally occurring background levels of arsenic, aluminum, iron and manganese in soil exceed risk-based screening levels. For arsenic, a known human carcinogen, many states in the U.S. rely upon naturally occurring background soil arsenic levels for cleanup decisions, with upper background concentrations ranging from 7 to 40 mg/kg. On the other hand, risk-based cleanup levels for arsenic used by some states can be up to two orders of magnitude lower than this range (Teaf et al., 2010). Reliance upon background concentration levels to support risk management decisions by some states and regions reflects a pragmatic approach that considers the possibility that resources spent on removing/treating marginally increased concentrations in soil might not produce a meaningful change in risk to human health (e.g., replacement soils may likely contain similar levels of arsenic).

Like risk-based concentrations for arsenic in soil, standard risk assessment practices applied to EO result in risk-based exposure concentrations for EO in air that are extremely low. In USEPA's Draft IRIS Toxicological Review for EO (USEPA, 2014), USEPA derived an inhalation unit risk value of 0.0018 per $\mu g/m^3$ based on epidemiology data for exposed workers. This unit risk value can be used to calculate a *de minimis* ($1 \times 10^{-6}$) risk-based concentration of 0.00031 ppb.

* Corresponding author.
*E-mail address:* ckirman@summittoxicology.com (C.R. Kirman).

http://dx.doi.org/10.1016/j.yrtph.2017.10.032
Received 3 October 2016; Received in revised form 8 September 2017; Accepted 26 October 2017
Available online 28 October 2017

Meanwhile, because of endogenous production of EO in the body, it is expected to be present in human blood at steady-state concentrations ranging from 0.04 to 017 nmol/L (Filser et al., 1992; Csanády et al., 2000). Based upon blood:air partition coefficients (Filser et al., 2013), these blood levels are predicted to correspond to exhaled breath concentrations of approximately 0.01–0.05 ppb. In short, standard risk assessment practices result in the calculation of risk-based concentrations for EO in air that are approximately two orders of magnitude lower than levels predicted in exhaled breath of humans with no exogenous EO exposure.

As an alternative to reliance on standard risk-based concentrations, a pragmatic approach is presented here for the derivation of endogenous equivalent (EE) values, which can be used to support risk assessment and risk management decisions for chemicals like EO that have both endogenous and exogenous exposure pathways.

## 2. Methods

Literature searches were conducted using publically available databases (Pubmed, TOXNET) using appropriate search terms (ethylene oxide, biomonitoring, biomarker, adducts, hemoglobin, protein, DNA, 2-hydroxyethyl valine, 2-hydroxyethyl guanine, blood, urine) to identify studies that provide biomarkers of exposure for EO in human populations to supplement those identified by Wu et al. (2011). The reference lists for review articles and recent publications were also used as secondary sources of relevant studies. Endogenous equivalents were determined for EO using the following steps: (1) Identify an appropriate biomarker of exposure; (2) Characterize the biomarker distribution in humans; and (3) Establish quantitative relationship between biomarker of exposure and external exposure. Resulting EE values reflect endogenous EO levels expressed in terms of equivalent exogenous exposure levels (e.g., ppm EO in air).

### 2.1. Identification of reliable biomarkers of exposure

Based upon animal studies, EO is well absorbed following exposures, and is rapidly distributed to all organs and tissues (USEPA, 2014). In tissues, EO is subject to metabolism by hydrolysis (yielding 1,2-ethanediol, hydroxyacetaldehyde, glycolic acid, glyoxylic acid, formic acid, oxalic acid, and carbon dioxide) or glutathione conjugation pathways (yielding mercapturic acids), both of which are considered to be detoxifying steps, and whose products are excreted in the urine. Some of the EO that is absorbed is eliminated unchanged in exhaled breath. As an epoxide, EO is capable of reacting with cellular macromolecules, including hemoglobin and DNA. Based upon a review of the published literature, potential biomarkers of exposure for EO include a consideration of the following: (1) EO in exhaled breath; (2) DNA adducts; and (3) hemoglobin adducts (Fig. 1). The primary advantage of using EO levels in exhaled breath for this assessment would be that it simplifies comparisons to risk-based concentrations for EO in air. Unfortunately, direct measurements for EO in exhaled breath are not available, and instead can only be estimated and/or predicted from toxicokinetic models (Filser et al., 2013; Csanády et al., 2000; Pennell and Brown, 2001).

As an alkylating agent, EO reacts with DNA to produce adducts including the major N7-2-hydroxyethylguanine (N7-HEG), which although a non-mutagenic adduct is a useful biomarker of exposure. N7-HEG adducts have been quantified in tissues in rodents exposed to ethylene, which is metabolized to EO in tissues (Wu et al., 1999; Walker et al., 2000; Rusyn et al., 2005) and rodents exposed to exogenous EO (van Sittert et al., 2000; Rusyn et al., 2005; Marsden et al., 2007, 2009; Zhang et al., 2015). N7-HEG adducts have also been used to characterize endogenous exposures in human lymphocytes (Wu et al., 1999). HEG levels in granulocytes have been reported as a useful biomarker in exposed workers (Yong et al., 2007). Similarly, N7-HEG adducts that have undergone repair via depurination are excreted in urine, which has been reported as a useful biomarker in smokers (Huang et al., 2008) and exposed workers (Huang et al., 2011). The primary disadvantages to relying upon N7-HEG adducts are: (1) the data are limited to just a few studies in humans; and (2) N7-adducts are chemically unstable (Boysen et al., 2009), and therefore may only be reflective of very recent exposures; and (3) N7-HEG adducts are not mutagenic and do not block DNA replication (Boysen et al., 2009; Philippin et al., 2014).

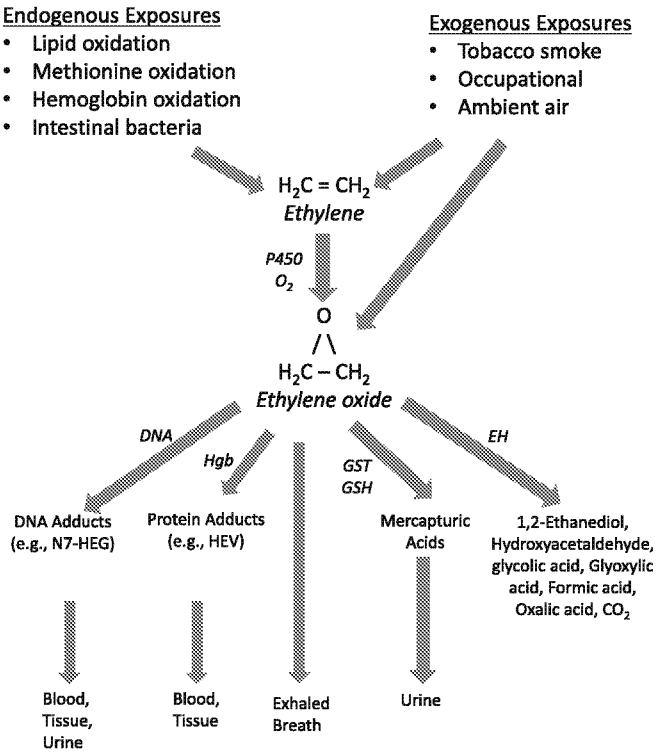


**Fig. 1.** Metabolism and Biomarkers for Ethylene Oxide. P450 = cytochrome P450; O2 = oxygen; DNA = deoxyribonucleic acid; Hgb = hemoglobin; EH = epoxide hydrolase; GSH = glutathione; GST = glutathione-S-transferase; N7-HEG = N7-hydroxyethyl guanine; HEV = N-2-hydroxyethyl valine.

Importantly, EO also forms adducts on the terminal valine of hemoglobin, N-(2-hydroxyethyl)-valine (HEV). These adducts are relatively stable, considerably more so than DNA adducts of EO (Wu et al., 2011), and are readily measurable in erythrocytes. Because of the half-life of EO hemoglobin is expected to reflect erythrocyte turnover in humans (approximately 120 days), HEV adducts reflect cumulative exposures to EO that occurred during the previous months. Since EO is widely distributed in the body, the levels of HEV in erythrocytes are expected to be proportionate to levels of HEV in other tissues (including tissues that are targets of EO toxicity), which in turn is expected to be proportionate to tissue exposures to free EO. HEV adducts have been well studied, including characterization by many studies in human populations with no known and/or negligible exogenous exposures (Table 1). EO and ethylene are both components of tobacco smoke. Accordingly, HEV adduct burdens are generally higher in smokers compared to non-smokers. HEV adducts have also been characterized in populations with significant exogenous exposures to EO, including smoking and occupational exposures (Table 2).

Based upon the review of the available biomarker data for EO, and based upon a consideration of data availability and stability of the biomarker, HEV adducts were selected as the most appropriate basis to support the derivation of EE values for EO.

### 2.2. Characterize biomarker distribution

The HEV adduct data in Tables 1 and 2 were used to support meta-

**Table 1**
Summary of HEV adduct data in human populations with no or negligible exogenous exposure to EO.

| Exposure Group | n | Adduct Burden (pmol/g Hgb) | | | | Reference |
|---|---|---|---|---|---|---|
| | | Mean | SD | Median | Range | |
| Nonsmoker/Control | 14 | 58 | 25.0 | NR | 27–106 | Törnqvist et al., 1986 |
| | 23 | 52.1 | 20.5 | NR | NR | Bailey et al., 1987 (as cited in Farmer et al., 1993) |
| | 7 | 32 | 15.0 | NR | NR | Tates et al., 1991 |
| | 6 | 117 | 123.0 | NR | NR | Tates et al., 1991 |
| | 4 | NR | NR | 20 | NR | Hagmar et al., 1991 |
| | 47 | 46.4 | 26.1 | NR | NR | Farmer et al., 1993 |
| | 10 | 63 | 20.0 | NR | 35.7–105.1 | Tavares et al., 1994 |
| | 29 | NR | NR | 30.3 | NR | Farmer et al., 1996a,b |
| | 21 | 62 | 40 | 50 | 22–176 | Müller et al., 1998 |
| | 23 | 22 | 24 | 19 | 6–49 | Boogaard et al., 1999 |
| | 74 | 17.0 | 21.1 | 9.1 | NR | Bono et al., 1999 |
| | 13 | 12.9 | 6.1 | NR | NR | Fennell et al., 2000 |
| | 5 | 40 | 89.4 | NR | NR | Yong et al., 2001 |
| | 21 | 13.6 | 5.8 | NR | NR | Thier et al., 2001 |
| | 172 | 29.4 | 33.6 | NR | NR | Bono et al., 2002 |
| | 10 | NR | NR | 17 | 9–150 | Schettgen et al., 2002 |
| | 20 | NR | NR | NR | 14–43 | Ball et al., 2004 |
| | 78 | 56.6 | 45.6 | NR | NR | Wu et al., 2004 |
| | 92 | NR | NR | 18.1 | 7.7–64.6 | Schettgen et al., 2010 |
| | 55 | 24 | 11.0 | 22 | 6.4–64 | von Stedingk et al., 2011 |
| Second Hand Smoke[a] | 28 | 16.6 | 23.3 | 10.7 | NR | Bono et al., 1999 |
| | 64 | 24.4 | 24.9 | NR | NR | Bono et al., 2003 |
| | 12 | NR | NR | 17.2 | 10.8–38.6 | Schettgen et al., 2010 |

[a] EO exposures from second hand smoke were deemed negligible since biomarker data were nominally lower, and statistically indistinguishable from corresponding data for unexposed subjects.

**Table 2**
Summary of HEV adduct data in human populations with significant exogenous exposures to EO.

| Exposure Group | n | Mean | SD | Median | Range | Reference |
|---|---|---|---|---|---|---|
| Smokers | 11 | 389 | 138.0 | | 217–690 | Törnqvist et al., 1986 |
| | 1 | 308 | 40.0 | | | Tates et al., 1991 |
| | 9 | 91 | 100.0 | | | Tates et al., 1991 |
| | 13 | 361 | 107.0 | | 218.9–559.6 | Tavares et al., 1994 |
| | 20 | NR | NR | 150.9 | | Farmer et al., 1996a,b |
| | 26 | 200 | 113.0 | | | Bailey et al., 1987; Farmer et al., 1993 |
| | 6 | 306 | 85 | 280 | 234–471 | Müller et al., 1998 |
| | 44 | 59.5 | 52.8 | 45.4 | | Bono et al., 1999 |
| | 18 | 242 | 131.5 | | | Fennell et al., 2000 |
| | 14 | 382 | 127.2 | | | Fennell et al., 2000 |
| | 38 | 19.2 | 7.4 | | | Thier et al., 2001 |
| | 124 | 99.6 | 78.2 | | | Bono et al., 2002 |
| | 60 | 197.7 | 145.2 | | | Wu et al., 2004 |
| | 10 | 254 | 185.6 | | | Wu et al., 2004 |
| | 6 | 400 | 140.0 | 410 | 210–560 | von Stedingk et al., 2011 |
| Occupational | 7 | 2215 | 2874.0 | 980 | 20–4600 | Farmer et al., 1996a,b |
| | 6 | 80 | 28.0 | | | Tates et al., 1991 |
| | 8 | 2720 | NR | | | Tates et al., 1991 |
| | 7 | 13,200 | 2550 | | | Tates et al., 1991 |
| | 15 | NR | NR | 50 | | Hagmar et al., 1991 |
| | 16 | NR | NR | 230 | | Hagmar et al., 1991 |
| | 8 | NR | NR | 1380 | | Hagmar et al., 1991 |
| | 4 | NR | NR | 10,000 | | Hagmar et al., 1991 |
| | 39 | 145 | NR | | | van Sittert et al., 1993 |
| | 41 | 238 | NR | | | van Sittert et al., 1993 |
| | 48 | 53 | NR | | | van Sittert et al., 1993 |
| | 9 | 15,472 | 7687 | | | Angerer et al., 1998 |
| | 3 | 1222 | 671 | | | Angerer et al., 1998 |
| | 20 | 92 | SE = 25 | 46 | 12–320 | Boogaard et al., 1999 |
| | 59 | ~13 NS; 18S | NR | | | Thier et al., 2001 |
| | 36 | 80 | SE = 10 | | | Yong et al., 2001 |
| | 17 | 160 | SE = 20 | | | Yong et al., 2001 |
| | 62 | NR | NR | 168 | 16–2353 | Schettgen et al., 2002 |
| | 18 | NR | NR | | 42–115 | Ball et al., 2004 |
| | 6 | 1210 | 777 | | 522–2396 | Bader et al., 2012 |

analyses for nonsmoking and smoking populations, respectively. Data for population samples with exposure to second hand smoke were also included in the analysis for nonsmokers since they were found to be statistically indistinguishable from companion data from nonsmokers (Bono et al., 2002; Schettgen et al., 2010). Studies were included in the meta-analysis if they met the following criteria: (1) assessed HEV levels in 5 or more individuals; (2) presented mean and standard deviation values for HEV levels; and (3) assessed HEV levels in individual with no known exogenous exposures (for characterizing background HEV levels in nonsmokers/passive smoke-exposed) or no known occupational exposures (for characterizing HEV levels in smokers). Based on these requirements, six data sets from Table 1 were excluded from the meta-analysis for nonsmokers (Hagmar et al., 1991; Farmer et al., 1996a,b; Schettgen et al., 2002, 2010; Ball et al., 2004), and two data sets from Table 2 were excluded from the meta-analysis for smokers (Tates et al., 1991; Farmer et al., 1996a,b). Together, the data sets for HEV used to support the meta-analyses include measurements in 661 nonsmokers/passive smoke-exposed and in 379 smokers.

The weighted means and standard deviations resulting from the meta-analyses were used to generate lognormal distributions for HEV levels for nonsmoking and smoking populations. Although raw data were not available to assess the underlying distribution of HEV adducts in human populations, HEV adducts were assumed to be lognormally distributed based upon a consideration of: (1) inspection of the mean value relative to the range detected for several data sets (i.e., lies closer to the minimum than the maximum); and (2) consistency with distributional analyses of hemoglobin adducts for other chemical alkylators reported in the literature (Brisson et al., 2014; Boysen et al., 2012).

### 2.3. Quantify relationship between biomarker and exogenous EO exposure

Although physiologically based pharmacokinetic models have been developed for EO (Filser et al., 2013; Csanády et al., 2000; Fennell and Brown, 2001), published correlations between exogenous EO exposures and HEV levels provide a much simpler means of relating internal and external doses of EO. The German research organization, Deutsche Forschungsgemeinschaft (DFG, 1994), established a correlation between HEV levels and occupational exposures to 500–2000 ppb EO (unfilled diamonds in Fig. 3). This correlation was based on measurements in exposed workers at factories of the German chemical industry (MAK, 2010). As validation for this predicted correlation, Angerer et al. (1998) collected environmental and HEV levels in 12 sterilization workers exposed to approximately 4200 ppb EO in air. HEV levels were determined twice over a 4-month interval, and are assumed to be representative of long-term, steady state levels. Data from Angerer et al. (1998) are depicted in Fig. 3 (filled diamond), and indicate excellent agreement with the theoretical correlation developed by DFG (1994). Based on fits of a linear, least-squares model to the combined data from these studies (Fig. 3; recreated from Fig. 2 of Angerer et al., 1998), this correlation can be expressed as the following equation for occupational exposures to EO:

$$\text{HEV (pmol/g)} = 3.74 * [\text{EO}]_{\text{occup}} \quad (1)$$

The correlation appears to be reasonably linear up to 4200 ppb EO for occupational exposures. The slope of this relationship can also be expressed as follows for 24-h, continuous exposures (by multiplying the slope in Eq. (1) by default factors of 20/10 $m^3$/day, and exposure frequencies of 365/250 to account for occupational ($[EO]_{occup}$) vs. continuous exposure ($[EO]_{cont}$) scenarios) (Fig. 3):

$$\text{HEV (pmol/g)} = 10.9 * [\text{EO}]_{\text{cont}} \quad (2)$$

Rearranging this equation, EE values are calculated as:

$$\text{EE (ppb EO, continuous)} = \text{HEV (pmol/g)}/10.9 \quad (3)$$

### 2.4. Statistics

Meta-analyses were performed using both fixed effect and random effect models using methods as described by Higgins and Thompson (2002). All calculations were performed in an Excel spreadsheet (version 15.16; Microsoft). Statistics and percentiles for the lognormal distribution generated for HEV values were used to calculate EE values for EO by extrapolating from internal dose measures to external exposures (e.g., ppb EO in air).

## 3. Results

Results of the meta-analyses for nonsmokers and smokers are provided in Table 3. For HEV data from nonsmokers, the fixed and random effects models returned very similar results for the modeled mean (20.5 vs 21.1 pmol/g) and standard deviation (14.0 vs. 14.6 pmol/g). The low values for I-squared indicates that nearly all of the total variation in the data is associated with within-study variation (i.e., very little between-study variation). In contrast, the fixed and random effects models applied to the HEV data from smokers returned very different results for the modeled mean (29.9 vs 205 pmol/g) and standard deviation (29.5 vs 193 pmol/g). The comparatively high value for I-squared for the fixed effects model indicates a large portion of the variation is associated with between-study variation. The large between-study variation is not surprising given differences in smoking habits for different populations. Because the data used to support the meta-analyses come from studies of differing methods and diverse populations (across geographic region, age, sex), the results for the random effects model were considered to be more appropriate than the fixed effect model for characterizing the distribution of HEV values in the two populations. The lognormal distribution for HEV values in nonsmokers is depicted in Fig. 2.

EE values derived for EO are summarized in Table 4. By analogy to characterizations of background metals in soil (USEPA, 2002), there are several options for identifying what level constitutes a "substantial difference" from background to warrant additional interest. These options include: (1) multiples of the mean background (exogenous) level; (2) multiples of the standard deviation for background levels; and (3) percentiles for the distribution of background levels. Using Eq. (3), EE values were calculated to range from 0.13 to 6.9 ppb EO in air for continuous exposure. The low end of EE levels (0.13 ppb in air) is associated with one tenth of the SD for the background variation, while the upper end of this range of EEs is associated with the 99th percentile of the lognormal distribution of the background HEV levels.

## 4. Discussion/Conclusion

EE values for EO were derived based upon a meta-analysis of data available from the published literature to characterize HEV levels in unexposed human populations (Table 1). These values can be used to support a variety of risk assessment and risk management decisions, several of which are discussed below.

With respect to risk assessment, existing dose-response assessments for EO do not take endogenous and non-occupation exposures to EO into consideration. For example, in USEPA's draft cancer risk assessment for EO, exposures to EO were assessed in terms of cumulative metric ppm-days. Specifically, for USEPA's evaluation of breast cancer, the mean cumulative exposures to EO for the first two deciles of the NIOSH cohort of exposed workers were 157 and 580 ppm-days (USEPA, 2014). Assuming a 45-year duration for occupational tenure, the mean EE value calculated for EO (2.9 ppb) corresponds to approximately a value of 140 ppm-days (occupational exposure), which is approximately equivalent to the lowest decile from the NIOSH cohort (i.e., endogenous exposure is roughly equivalent to exogenous exposure from the cohort occupationally exposed to EO). Furthermore, because cigarette smoking was not considered in NIOSH workers, non-

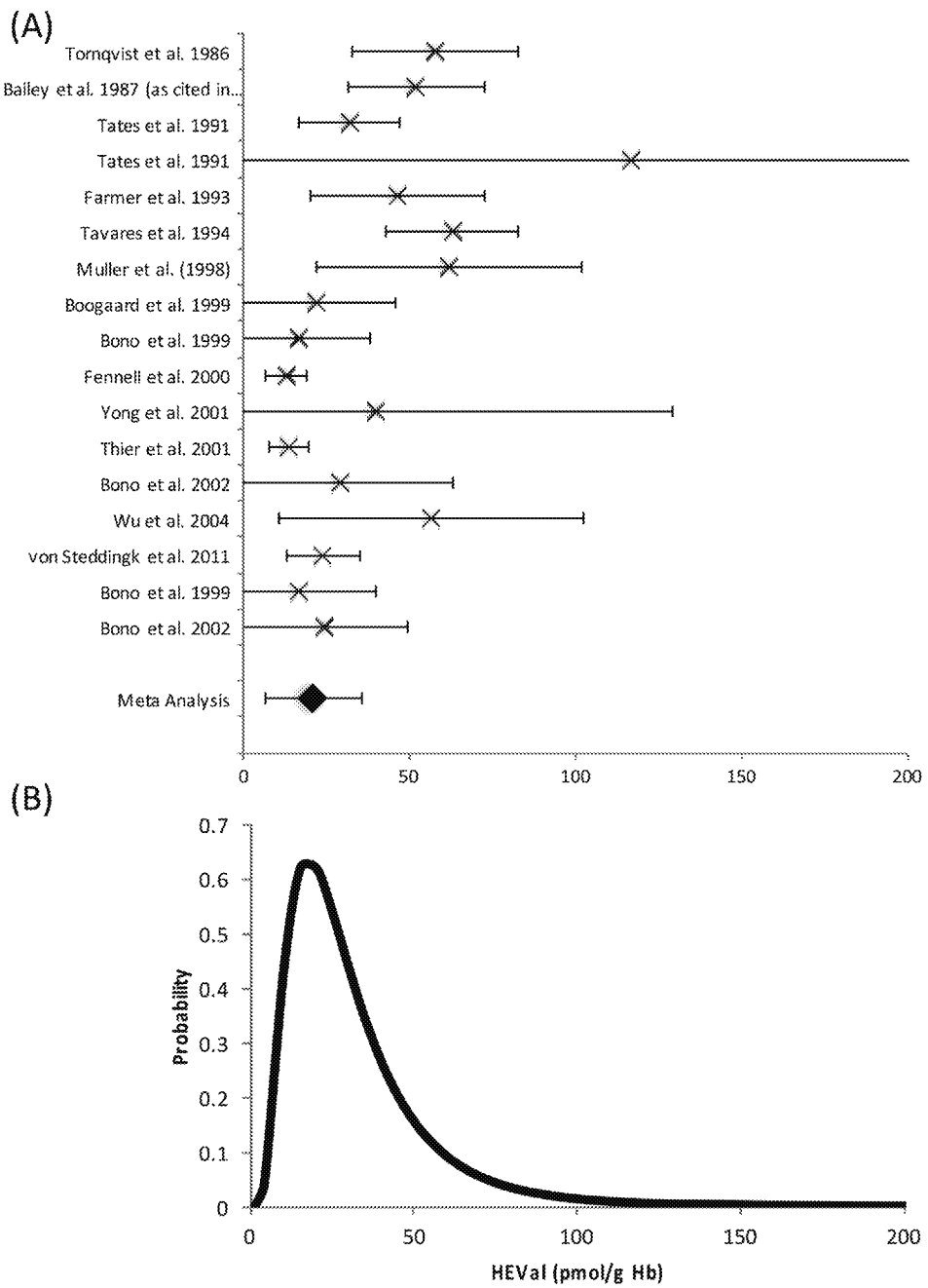


**Fig. 2.** Characterization of HEV Adducts (pmol/g Hb) in Unexposed Populations: (A) Mean (X) and SD (bars) reported in individual studies; (B) Lognormal probability density function for the combined data set.

occupational EO exposures could be up to an order of magnitude higher (random effects model predictions of 205 vs 21 pmol/g for smokers and nonsmokers, respectively), depending upon smoking prevalence, which would bring the non-occupational exposures (endogenous EO + EO from smoking) near the value of the second decile. This comparison indicates that there is considerably more uncertainty and a lack of precision in the exposure estimates for EO when total EO exposures (endogenous + smoking + occupational) are considered. In the highest exposure deciles for the NIOSH cohort (e.g., greater than 10,000 ppm-days), total EO exposure is dominated by the occupational component, and therefore, ignoring the other components does not impact the dose-response modeling in the high-exposure region. However, this is not the case for the lowest deciles, which are particularly important for estimating the point of departure used to quantify cancer potency, and the potential underestimation of EO exposure may result in an overestimation of EO's cancer potency. Additionally, EE values for EO could be used to support alternative exposure metrics in the dose-response assessment. For example, using the epidemiology data the relative risk of cancer could be assessed in terms of relative dose (e.g., calculated as total EO exposure divided by the mean EE value).

The EE values for EO can be used to check key assumptions in the EO cancer risk assessment. Based upon the weighted means from the meta-analyses (205 vs. 21 pmol/g), smokers experience an approximate an order of magnitude higher internal EO exposure compared to nonsmokers. Accordingly, assuming a default assumption of low-dose linearity for the EO dose-response relationship, smokers would be expected to experience a detectable increase in cancer endpoints on which EO cancer potency estimates have been derived (i.e., lymphohematopoietic, breast cancers). However, this prediction is not supported by observation. With respect to lymphohematopoietic cancers, for only one subtype, acute myeloid leukemia (AML), has sufficient evidence of a causal relationship with smoking been inferred (USDHHS, 2014). Previously, other lymphohematopoietic cancers such as lymphomas and multiple myeloma were omitted from the Surgeon General's report

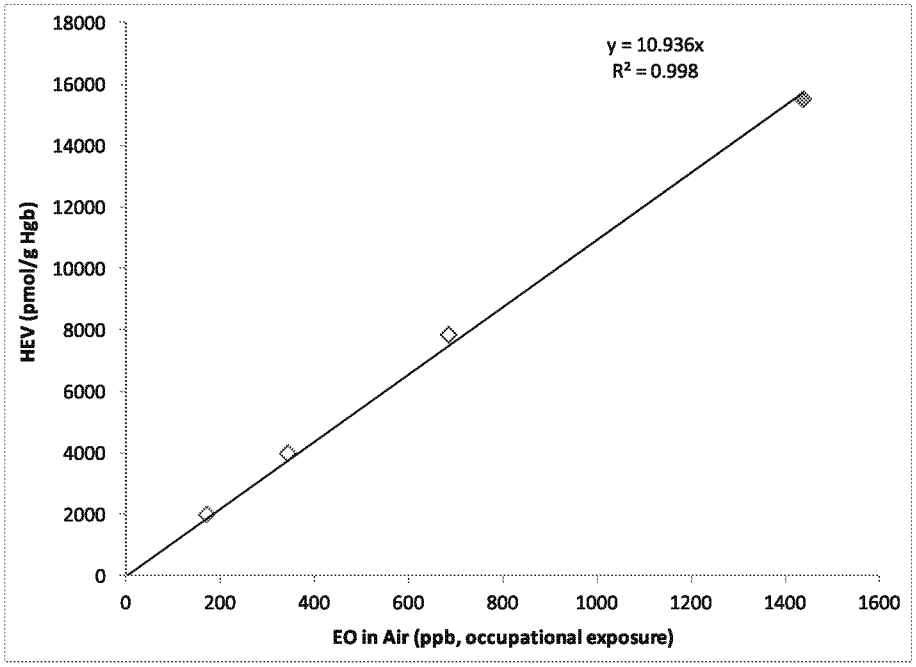


**Fig. 3.** Correlation between HEV Adducts (pmol/g Hb) and occupational exposures to EO (ppm in air). Unfilled Diamonds = data point (DFG, 1994); Solid Diamond = data point (Angerer et al., 1998); Solid line = linear regression.

**Table 3**
Meta-analyses results for nonsmoker/passive smoker and smoker data sets.

| Parameter | HEV in Nonsmokers/Passive Smoke-Exposed | | HEV in Smokers | |
|---|---|---|---|---|
| | Fixed Effects Model | Random Effects Model | Fixed Effects Model | Random Effects Model |
| Weighted mean (pmol/g) | 20.5 | 21.1 | 29.9 | 205.4 |
| SD (pmol/g) | 14.0 | 14.6 | 29.5 | 193.3 |
| Q | 16.3 | 15.8 | 52.8 | 8.3 |
| df | 16.0 | 16.0 | 12.0 | 12.0 |
| p-value | 0.4 | 0.5 | 0.0 | 0.8 |
| Tau-sq | 4.5 | 0.0 | 17,257 | 0.0 |
| I-squared | 1.9 | 0 | 77.3 | 0 |

**Table 4**
Endogenous equivalent values for ethylene oxide.

| Basis | Multiplier or Percentile | Hemoglobin Adduction Burden (pmol/g) | Endogenous Equivalent Concentration in Air (ppb, Continuous)[a] |
|---|---|---|---|
| Multiples of mean (21.1 pmol/g) | 0.1 | 2.1 | 0.19 |
| | 0.25 | 5.3 | 0.48 |
| | 0.5 | 10.5 | 1.0 |
| | 1 | 21.1 | 1.9 |
| | 2 | 42.1 | 3.9 |
| | 3 | 63.2 | 5.8 |
| Multiples of SD (14.6 pmol/g) | 0.1 | 1.5 | 0.13 |
| | 0.25 | 3.6 | 0.33 |
| | 0.5 | 7.3 | 0.67 |
| | 1 | 14.6 | 1.3 |
| | 2 | 29.2 | 2.7 |
| | 3 | 43.8 | 4.0 |
| Percentile for Lognormal Distribution | 0 | 0 | 0.0 |
| | 0.01 | 4.0 | 0.37 |
| | 0.05 | 6.1 | 0.56 |
| | 0.1 | 7.7 | 0.71 |
| | 0.25 | 11.3 | 1.0 |
| | 0.5 | 17.3 | 1.6 |
| | 0.75 | 26.4 | 2.4 |
| | 0.9 | 38.8 | 3.5 |
| | 0.95 | 48.7 | 4.5 |
| | 0.99 | 74.9 | 6.9 |

[a] Calculated using Eq. (3): EE = HEV (pmol/g)/10.9.

because they have not been linked to smoking (USDHHS, 2004). The relationship for AML and smoking is generally attributed to the presence of known leukemogens in tobacco smoke (Thomas and Chelghoum, 2004). Perhaps, more importantly, when the SMRs for AML in NIOSH workers exposed to EO are specifically inspected, these are not increased. On the contrary, a statistically significant negative slope was observed for the relationship between cumulative exposures to EO and AML in the NIOSH cohort (Valdez-Flores et al., 2010). With respect to breast cancer, the evidence for a causal relationship with smoking is not strong, and is instead considered to be suggestive, but not sufficient (USDHHS, 2014). Because the predictions of risk from observations made in highly exposed populations (i.e., occupational cohorts) do not appear to be supported by observations in moderately exposed populations (i.e., smokers), the results of this comparison suggests that some of the underlying assumptions used in the cancer potency estimate for EO should be revisited prior to their application to predict risk in populations with low exogenous exposures to EO (i.e., nonsmoking, general public).

With respect to risk management decisions, there are two general approaches to managing the risks associated with chemicals that have both exogenous and endogenous exposure pathways. Under a precautionary approach, it is assumed that endogenous exposures contribute to background risk either because there is no threshold for the endpoint of concern, or because endogenous exposures are sufficiently near or exceed any such threshold. Under a pragmatic approach, can be assumed that endogenous exposures reflect a stressor to which species have evolved and adapted over millions of years, and for which there is considerable inter-individual and temporal variation. This variation creates a signal-to-noise issue when exogenous exposures fall well below those consistent with endogenous exposures. In such cases, small exogenous exposures may not contribute to total exposure or to potential effects in a biologically meaningful way. The validity of either low-dose assumption (linear or threshold), which in turn affects the acceptability of the precautionary and pragmatic approaches, should take careful consideration of the underlying mode(s) of action for carcinogenic effects. A review of mode of action information for EO carcinogenicity is beyond the scope of this manuscript. Additionally,

bounds can be placed on the magnitude of any linear estimate of cancer potency for EO using the "bottom-up" approach described by Starr and Swenberg (2013).

Returning to the example of managing the risks of arsenic in soil, these two approaches are readily apparent in contrasting states in the U.S. that rely upon risk-based concentrations (precautionary approach) with those states that rely upon regional background levels (pragmatic approach) (Teaf et al., 2010). In the same way, risk managers adopting a precautionary approach can continue to rely upon risk-based concentrations derived for EO, which as stated above, can lead to regulating EO at very low concentrations (e.g., 0.0003 ppb in air), which corresponds to approximately 0.01% of the mean endogenous exposure level. The approach described in this work in deriving EE values is intended to help risk managers in search of a more pragmatic, science-based approach to managing the potential risks from EO exposure, particularly when the potential health benefits of its use (e.g., disinfection of medical equipment) are considered. For these risk managers, the range of EE values for EO (0.13–6.9 ppb; Table 2) may be considered sufficiently protective of human health. The approach described herein is generally applicable to other agents (e.g., acetaldehyde, ammonia, cyanide, formaldehyde, hydrogen sulfide, methanol) for risk management requires a consideration of both exogenous and endogenous exposures.

## Acknowledgments and Conflict of interest statement

The analysis presented here was funded by the Ethylene Oxide Panel of the American Chemistry Council (contract 5478).

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.10.032.

## References

Angerer, J., Bader, M., Krämer, A., 1998. Ambient and biochemical effect monitoring of workers exposed to ethylene oxide. Int. Arch. Occup. Environ. Health 71 (1), 14–18.
Bader, M., Will, W., Frey, G., Nasterlack, M., 2012. Analysis of protein adducts as biomarkers of short-term exposure to ethylene oxide and results of follow-up biomonitoring. Arh. Hig. Rada Toksikol. 63 (2), 107–115.
Bailey, E., Farmer, P.B., Shuker, D.E., 1987. Estimation of exposure to alkylating carcinogens by the GC-MS determination of adducts to hemoglobin and nucleic acid bases in urine. Arch. Toxicol. 60 (1–3), 187–191.
Ball, L., Jones, A., Boogaard, P., Will, W., Aston, P., 2004. Development of a competitive immunoassay for the determination of N-(3-hydroxyethyl)valine adducts in human haemoglobin and its application in biological monitoring. Biomarkers 9 (6), 407–417.
Bono, R., Vincenti, M., Saglia, U., Pignata, C., Russo, R., Gilli, G., 2002. Tobacco smoke and formation of N-(3-hydroxyethyl)valine in human hemoglobin. Arch. Environ. Health 57 (5), 416–421.
Bono, R., Vincenti, M., Meineri, V., Pignata, C., Saglia, U., Giachino, O., Soursatone, E., 1999. Formation of N-(2-hydroxyethyl)valine due to exposure to ethylene oxide via tobacco smoke: a risk factor for onset of cancer. Environ. Res. 81 (1), 62–71.
Boogaard, P.J., Rocchi, P.S., van Sittert, N.J., 1999. Biomonitoring of exposure to ethylene oxide and propylene oxide by determination of hemoglobin adducts: correlations between airborne exposure and adduct levels. Int. Arch. Occup. Environ. Health 72 (3), 142–150.
Boysen, G., Pachkowski, B.F., Nakamura, J., Swenberg, J.A., 2009. The formation and biological significance of N7-guanine adducts. Mutat. Res. 678 (2), 76–94.
Boysen, G., Georgieva, N.I., Borderat, N.K., Sram, R.J., Vacek, P., Albertini, R.J., Swenberg, J.A., 2012 Jan. Formation of 1,2:3,4-diepoxybutane-specific hemoglobin adducts in 1,3-butadiene exposed workers. Toxicol. Sci. 125 (1), 30–40.
Brisson, B., Ayotte, P., Normandin, L., Gaudreau, E., Bienvenu, J.F., Fennell, T.R., Blanchet, C., Phaneuf, D., Lapointe, C., Bonvalot, Y., Gagné, M., Courteau, M., Snyder, R.W., Bouchard, M., 2014. Relation between dietary acrylamide exposure and biomarkers of internal dose in Canadian teenagers. J. Expo. Sci. Environ. Epidemiol. 24, 215–221.
Clemens, M.R., Einsele, H., Frank, H., Remmer, H., Waller, H.D., 1983. Volatile hydrocarbons from hydrogen peroxide-induced lipid peroxidation of erythrocytes and their cell compounds. Biochem. Pharmacol. 32, 3877–3878.
Csanády, G.A., Denk, B., Putz, C., Kreuzer, P.E., Kessler, W., Baur, C., Gargas, M.L., Filser, J.G., 2000. A physiological toxicokinetic model for exogenous and endogenous ethylene and ethylene oxide in rat, mouse, and human: formation of 2-hydroxyethyl adducts with hemoglobin and DNA. Toxicol. Appl. Pharmacol. 165 (1), 1–26.
DFG 1994. Deutsche Forschungsgemeinschaft. Luftanalysen. Senatskommission zur Prüfung gesundheitsschadlicher Arbeitsstoffe, 9. Lieferung. VCH, Weinheim.
Farmer, P.B., Cordero, R., Autrup, H., 1996a. Monitoring human exposure to 2-hydroxyethylating carcinogens. Environ. Health Perspect. 104 (Suppl. 3), 449–452.
Farmer, P.B., Bailey, E., Naylor, S., Anderson, D., Brooks, A., Cushnir, J., Lamb, J.H., Sepai, O., Tang, Y.S., 1993. Identification of endogenous electrophiles by means of mass spectrometric determination of protein and DNA adducts. Environ. Health Perspect. 99, 19–24.
Farmer, P.B., Sepai, O., Lawrence, R., Autrup, H., Sabro Nielsen, P., Vestergård, A.B., Waters, R., Leuratti, C., Jones, N.J., Stone, J., Baan, R.A., van Delft, J.H., Steenwinkel, M.J., Kyrtopoulos, S.A., Souliotis, V.L., Theodorakopoulos, N., Bacalis, N.C., Natarajan, A.T., Tates, A.D., Haugen, A., Andreassen, A., Ovrebø, S., Shuker, D.E., Amamng, K.S., Castelain, P., et al., 1996b. Biomonitoring human exposure to environmental carcinogenic chemicals. Mutagenesis 11 (4), 365–381.
Fennell, T.R., MacNeela, J.P., Morris, R.W., Watson, M., Thompson, C.L., Bell, D.A., 2000. Hemoglobin adducts from acrylonitrile and ethylene oxide in cigarette smokers: effects of glutathione S-transferase T1-null and M1-null genotypes. Cancer Epidemiol. Biomarkers Prev. 9 (7), 705–712.
Fennell, T.R., Brown, C.D., 2001. A physiologically based pharmacokinetic model for ethylene oxide in mouse, rat, and human. Toxicol. Appl. Pharmacol. 173 (3), 161–175.
Filser, J.G., Kessler, W., Artati, A., Erbach, E., Faller, T., Kreuzer, P.E., Li, Q., Lichtmannegger, J., Numtip, W., Klein, D., Putz, C., Sender, B., Csanády, G.A., 2013. Ethylene oxide in blood of ethylene-exposed B6C3F1 mice, Fischer 344 rats, and humans. Toxicol. Sci. 136 (2), 344–358.
Filser, J.G., Denk, B., Törnqvist, M., Kessler, W., Ehrenberg, L., 1992. Pharmacokinetics of ethylene in man: body burden with ethylene oxide and hydroxyethylation of hemoglobin due to endogenous and environmental ethylene. Arch. Toxicol. 66 (3), 157–163.
Hagmar, L., Welinder, H., Lindén, K., Attewell, R., Osterman-Golkar, S., Törnqvist, M., 1991. An epidemiological study of cancer risk among workers exposed to ethylene oxide using hemoglobin adducts to validate environmental exposure assessments. Int. Arch. Occup. Environ. Health 63 (4), 271–277.
Higgins, J.P.T., Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. Stat. Med. 21, 1539–1558.
Huang, C.C., Wu, C.F., Shih, W.C., Chen, M.F., Chen, C.Y., Chien, Y.C., Liou, S.H., Chiang, S.Y., Wu, K.Y., 2011. Comparative analysis of urinary N7-(2-hydroxyethyl)guanine for ethylene oxide- and non-exposed workers. Toxicol. Lett. 202 (3), 237–243.
Huang, C.C., Shih, W.C., Wu, C.F., Chen, M.F., Chen, Y.L., Lin, Y.H., Wu, K.Y., 2008. Rapid and sensitive on-line liquid chromatographic/tandem mass spectrometric determination of an ethylene oxide-DNA adduct, N7-(2-hydroxyethyl)guanine, in urine of nonsmokers. Rapid Commun. Mass Spectrom. 22 (5), 706–710.
Kessler, W., Remmer, H., 1990. Generation of volatile hydrocarbons from amino acids and proteins by an iron/ascorbate/GSH system. Biochem. Pharmacol. 39, 1347–1351.
Liberman, M., Mapson, L.W., 1964. Genesis and biogenesis of ethylene. Nature 204, 343–345.
Lynch, D.W., Lewis, T.R., Moorman, W.J., Burg, J.R., Groth, D.H., Khan, A., Ackerman, L.J., Cockrell, B.Y., 1984. Carcinogenic and toxicologic effects of inhaled ethylene oxide and propylene oxide in F344 rats. Toxicol. Appl. Pharmacol. 76 (1), 69–84.
MAK, 2010. Addendum (BAT Value Documentation, 2010). Ethylene oxide.
Marsden, D.A., Jones, D.J., Britton, R.G., Ognibene, T., Ubick, E., Johnson, G.E., Farmer, P.B., Brown, K., 2009. Dose-response relationships for N7-(2-hydroxyethyl)guanine induced by low-dose [14C]ethylene oxide: evidence for a novel mechanism of endogenous adduct formation. Cancer Res. 69 (7), 3052–3059.
Marsden, D.A., Jones, D.J., Lamb, J.H., Tompkins, E.M., Farmer, P.B., Brown, K., 2007. Determination of endogenous and exogenously derived N7-(2-hydroxyethyl)guanine adducts in ethylene oxide-treated rats. Chem. Res. Toxicol. 20 (2), 290–299.
Müller, M., Krämer, A., Angerer, J., Hallier, E., 1998. Ethylene oxide-protein adduct formation in humans: influence of glutathione-S-transferase polymorphisms. Int. Arch. Occup. Environ. Health 71 (7), 499–502.
NTP, 1987. NTP toxicology and carcinogenesis studies of ethylene oxide (CAS No. 75-21-8) in B6C3F1 mice (Inhalation studies). Natl. Toxicol. Program Tech. Rep. Ser. 326, 1–114 1987 Nov.
Philippin, G., Cadet, J., Gasparutto, D., Mazon, G., Fuchs, R.P., 2014. Ethylene oxide and propylene oxide derived N7-alkylguanine adducts are bypassed accurately in vivo. DNA Repair (Amst) 22, 133–136.
Rusyn, I., Asakura, S., Li, Y., Kosyk, O., Koc, H., Nakamura, J., Upton, P.B., Swenberg, J.A., 2005. Effects of ethylene oxide and ethylene inhalation on DNA adducts, apurinic/apyrimidinic sites and expression of base excision DNA repair genes in rat brain, spleen, and liver. DNA Repair (Amst) 4 (10), 1099–1110.
Sagai, M., Ichinose, T., 1980. Age-related changes in lipid peroxidation measured by ethane, propane, butane, and pentane in respired gas of rats. Life Sci. 27, 731–738.
Schettgen, T., Broding, H.C., Angerer, J., Drexler, H., 2002. Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide-biomarkers in occupational and environmental medicine. Toxicol. Lett. 134 (1–3), 65–70.
Schettgen, T., Müller, J., Fromme, H., Angerer, J., 2010. Simultaneous quantification of haemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile, acrylamide and glycidamide in human blood by isotope-dilution GC/NCI-MS/MS. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 878 (27), 2467–2473.
Shen, J., Kessler, W., Denk, B., Filser, J.G., 1989. Metabolism and endogenous production of ethylene in rat and man. Arch. Toxicol. 13 (Suppl. 1), 237–239.
Snellings, W.M., Weil, C.S., Maronpot, R.R., 1984. A two-year inhalation study of the carcinogenic potential of ethylene oxide in Fischer 344 rats. Toxicol. Appl. Pharmacol. 75 (1), 105–117.
Starr, T.B., Swenberg, J.A., 2013. A novel bottom-up approach to bounding low-dose human cancer risks from chemical exposures. Regul. Toxicol. Pharmacol. 65 (3),

311–315.
Steenland, K., Whelan, E., Deddens, J., Stayner, L., Ward, E., 2003. Ethylene oxide and breast cancer incidence in a cohort study of 7576 women (United States). Cancer Causes Control 14 (6), 531–539 2003 Aug.
Steenland, K., Stayner, L., Deddens, J., 2004. Mortality analyses in a cohort of 18 235 ethylene oxide exposed workers: follow up extended from 1987 to 1998. Occup. Environ. Med. 61 (1), 2–7.
Tates, A.D., Grummt, T., Törnqvist, M., Farmer, P.B., van Dam, F.J., van Mossel, H., Schoemaker, H.M., Osterman-Golkar, S., Gebel, C., Tang, Y.S., et al., 1991. Biological and chemical monitoring of occupational exposure to ethylene oxide. Mutat. Res. 250 (1–2), 483–497.
Tavares, R., Ramos, P., Palminha, J., Bispo, M.A., Paz, I., Bras, A., Rueff, J., Farmer, P.B., Bailey, E., 1994. Transplacental exposure to genotoxins. Evaluation in haemoglobin of hydroxyethylvaline adduct levels in smoking and non-smoking mothers and their newborns. Carcinogenesis 15 (6), 1271–1274.
Teaf, C.M., Covert, D.J., Teaf, P.A., Page, E., Stark, M.J., 2010. Arsenic cleanup criteria for soils in the US and abroad: comparing guidelines and understanding inconsistencies. In: Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy, vol. 15. pp. 93–102 Article 10.
Teta, M.J., Sielken Jr., R.L., Valdez-Flores, C., 1999 Dec. Ethylene oxide cancer risk assessment based on epidemiological data: application of revised regulatory guidelines. Risk Anal. 19 (6), 1135–1155.
Thier, R., Balkenhol, H., Lewalter, J., Selinski, S., Dommermuth, A., Bolt, H.M., 2001. Influence of polymorphisms of the human glutathione transferases and cytochrome P450 2E1 enzyme on the metabolism and toxicity of ethylene oxide and acrylonitrile. Mutat. Res. 482 (1–2), 41–46.
Thomas, X., Chelghoum, Y., 2004 Jun. Cigarette smoking and acute leukemia. Leuk. Lymphoma 45 (6), 1103–1109.
Törnqvist, M., Osterman-Golkar, S., Kautiainen, A., Jensen, S., Farmer, P.B., Ehrenberg, L., 1986. Tissue doses of ethylene oxide in cigarette smokers determined from adduct levels in hemoglobin. Carcinogenesis 7 (9), 1519–1521.
Törnqvist, M., Gustafson, B., Kautiainen, A., Harms-Ringdahl, M., Granath, F., Ehrenberg, L., 1989. Unsaturated lipids and intestinal bacteria as sources of endogenous production of ethylene and ethylene oxide. Carcinogenesis 10, 39–41.
USDHHS, 2004. The Health Consequences of Smoking. A Report of the Surgeon General. U.S. Department of Health and Human Services. Atlanta, GA.
USDHHS, 2014. The Health Consequences of Smoking – 50 Years of Progress. A Report of the Surgeon General. U.S. Department of Health and Human Services, Atlanta, GA.
USEPA, 2014. Evaluation of the Inhalation Carcinogenicity of Ethylene Oxide (CASRN 75-21-8) In Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-14/194a.
USEPA, 2002. Role of Background in the CERCLA Cleanup Program. U.S. Environmental Protection Agency OSWER 9285.6-07P.
Valdez-Flores, C., Sielken Jr., R.L., Teta, M.J., 2010. Quantitative cancer risk assessment based on NIOSH and UCC epidemiological data for workers exposed to ethylene oxide. Regul. Toxicol. Pharmacol. 56 (3), 313–320.
van Sittert, N.J., Beulink, G.D., van Vliet, E.W., van der Waal, H., 1992. Monitoring occupational exposure to ethylene oxide by the determination of hemoglobin adducts. Environ. Health Perspect. 99, 217–220.
van Sittert, N.J., Boogaard, P.J., Natarajan, A.T., Tates, A.D., Ehrenberg, L.G., Törnqvist, M.A., 2000. Formation of DNA adducts and induction of mutagenic effects in rats following 4 weeks inhalation exposure to ethylene oxide as a basis for cancer risk assessment. Mutat. Res. 447 (1), 27–48.
von Stedingk, H., Viksström, A.C., Rydberg, P., Pedersen, M., Nielsen, J.K., Segerbäck, D., Knudsen, L.E., Törnqvist, M., 2011. Analysis of hemoglobin adducts from acrylamide, glycidamide, and ethylene oxide in paired mother/cord blood samples from Denmark. Chem. Res. Toxicol. 24 (11), 1957–1965.
Walker, V.E., Wu, K.Y., Upton, P.B., Ranasinghe, A., Scheller, N., Cho, M.H., Vergnes, J.S., Skopek, T.R., Swenberg, J.A., 2000. Biomarkers of exposure and effect as indicators of potential carcinogenic risk arising from in vivo metabolism of ethylene to ethylene oxide. Carcinogenesis 21 (9), 1661–1669.
Wu, K.Y., Ranasinghe, A., Upton, P.B., Walker, V.E., Swenberg, J.A., 1999. Molecular dosimetry of endogenous and ethylene oxide-induced N7-(2-hydroxyethyl) guanine formation in tissues of rodents. Carcinogenesis 20 (9), 1787–1792.
Wu, K.Y., Chiang, S.Y., Huang, T.H., Tseng, Y.S., Chen, Y.L., Ruo, H.W., Hsieh, C.L., 2004. Formation of N-(2-hydroxyethyl)valine in human hemoglobin-effect of lifestyle factors. Mutat. Res. 559 (1–2), 73–82.
Wu, K.Y., Chiang, S.Y., Shih, W.C., Huang, C.C., Chen, M.F., Swenberg, J.A., 2011. The application of mass spectrometry in molecular dosimetry: ethylene oxide as an example. Mass Spectrom. Rev. 30 (5), 733–756.
Yong, L.C., Schulte, P.A., Kao, C.Y., Giese, R.W., Boeniger, M.F., Strauss, G.H., Petersen, M.R., Wiencke, J.K., 2007. DNA adducts in granulocytes of hospital workers exposed to ethylene oxide. Am. J. Ind. Med. 50 (4), 293–302.
Yong, L.C., Schulte, P.A., Wiencke, J.K., Boeniger, M.F., Connally, L.B., Walker, J.T., Whelan, E.A., Ward, E.M., 2001. Hemoglobin adducts and sister chromatid exchanges in hospital workers exposed to ethylene oxide: effects of glutathione S-transferase T1 and M1 genotypes. Cancer Epidemiol. Biomarkers Prev. 10 (5), 539–550.
Zhang, F., Bartels, M.J., LeBaron, M.J., Schisler, M.R., Jeong, Y.C., Goliapudi, B.B., Moore, N.P., 2015. LC-MS/MS simultaneous quantitation of 2-hydroxyethylated, oxidative, and unmodified DNA nucleosides in DNA isolated from tissues of mice after exposure to ethylene oxide. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 976–977, 33–48 2015 Jan 22.